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The interaction of arsenic (As) and chromium (Cr) influences growth and antioxidant status in tossa jute (*Corchorus olitorius*)

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Abstract

Co-occurrence of arsenic (As) and chromium (Cr) is observed worldwide. However, their combined effects on plant physiology are not well documented. This experiment was designed to study both individual and combined phytotoxicity of arsenic (As) and chromium (Cr) in jute in terms of plant growth, net photosynthesis, chlorophyll content, oxidative stress and antioxidant enzyme activities. Two jute varieties, O-795 (Cr-tolerant) and O-9897 (Cr-sensitive), were grown under various level of As and/or Cr in pots under greenhouse condition. As or Cr stress significantly decreased plant growth, chlorophyll content, chlorophyll florescence (Fv/Fm), photosynthetic rate (Pn) and caused oxidative damage compared to control, indicated by increased MDA and H_2O_2 contents. The activities of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT), were dramatically increased in both varieties compared to control. Moreover, less severe inhibition of plant growth and oxidative damage was observed in O-795 than in O-9897 indicating variety O-795 had more efficient defense system to mitigate heavy metal induced oxidative stress. The combined stress of low level of As (50 mg kg^{-1}) plus Cr treatment caused less inhibition of plant growth and alleviated oxidative stress, in both varieties compared to Cr stress alone. In contrast, high level (100 mg kg^{-1}) As plus Cr caused a further decreased plant growth and chlorophyll content, increased MDA and H_2O_2 contents as well as antioxidative enzymes activities significantly ($P \leq 0.05$). These results suggest that the combined toxicity of low level As plus Cr in jute is lower than that of individual Cr or As. As exposure up to 50 mg kg^{-1} is supposed to be useful in mitigating Cr toxicity and more than 50 mg kg^{-1} had the synergistic toxic effect.

Keywords: arsenic; antioxidative enzyme; chromium; jute; oxidative stress.
Abbreviations: SOD_superoxide dismutase; POD_peroxidase; CAT_catalase; APX_ascorbate peroxidase; ROS_reactive oxygen species.

Introduction

In recent years, heavy metal pollution in soil has gained major concern due to its negative impact on agricultural production and human health (Rahaman et al., 2014; Bonanno, 2012). Chromium (Cr) and arsenic (As) rank 7 and 1, respectively among the top 20 hazardous substances (ATSDR, 2005). These are released to the environment through anthropogenic activities such as mining, agricultural activities and petroleum refineries (Martinez-Sanchez et al., 2011; Kabata-Pendias, 2011) and reached to toxic levels (Sridhar et al., 2011). Chromium, a toxic heavy metal, can cause serious problems to microbes, plants and animals even at trace concentrations, and can be highly toxic to human being as well through its bioaccumulation in the food chain (Tiwari et al., 2013). Considerable amount of Cr, Cd, As, Se and Ni exist because of geochemical and artificial activities in soil (Deverel and Fujii, 1990). Moreover organic fertilizers such as biosolids and phosphorus fertilizers contain considerable amount of Cr (Bini et al., 2000), which added more Cr to agricultural soils. Furthermore, both Cr and As are released from wood preservative (chromated-copper-arsenate, CCA) to the environment. CCA readily leaches from wood, resulting in the extensive spread soil contamination by As and Cr (Hingston et al., 2001; Kumpiene et al., 2008). Arsenic is also a non-essential element for plants and animals which can enter into the environment through natural as well as geological activities. It inhibits plant growth and interferes with various metabolic processes that cause physiological and morphological disorders particularly in higher doses (Mokgalaka-Matlala et al., 2008). Since As is carcinogenic, its presence in agricultural soils are of great environmental concern (Fayiga and Ma, 2006). Exposure to the heavy metal resulted in the disturbance in redox homeostasis in plant and induces reactive oxygen species (ROS) (Sharmin et al., 2012; Sinam et al., 2012; Gomes et al., 2013; Yu et al., 2013). The ROS (hydroxyl radicals; OH^-, superoxide radicals; O_2^-, hydrogenperoxide; H_2O_2) has a number of negative effects on plants such as damage of cell membranes, chloroplast pigments, lipids, nucleic acids and proteins (Molassiotis et al., 2006; Gunes et al., 2007). To cope with the oxidative damage, plants have evolved with an extensive network of antioxidant enzymes, such as superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione reductase and low molecular weight antioxidants, such as ascorbate, glutathione and α-tocopherol (Sairam et al., 2005; Gunes et al., 2007; Sinha et al., 2010). If the balance between ROS production and antioxidant system activity is disrupted, there will be a deleterious level of ROS accumulation in plants. Arsenic toxicity can cause oxidative damage due to increasing ROS and lipid peroxidation in chickpea (Gunes et al., 2009), fern species (Srivastava et al., 2005), rice (Dwivedi et al., 2010). The activities of antioxidative enzymes (SOD, POD, APX, CAT, GR) are markedly increased in plants under As stress (Jin et al., 2010). Under Cr stress, ROS are accumulated in plants (Sinam et al., 2012). Consequently, an increased level of H_2O_2 and MDA are noticed in plants while encountered with higher Cr concentration (Gallego et al., 2002; Pandey et al., 2005). SOD, POD, GR, CAT and APX activities are altered at higher Cr concentration in plants. Tolerance to As and Cr differs greatly among plant species and among genotypes within a species. In soil, As and Cr primarily exist as inorganic oxyanions. Plants accumulate them (Sridhar et al., 2007), and face growth reduction, alteration of mineral nutrition metabolism and oxidative stress (Raj et al., 2011). In nature, heavy metals occur together as contaminants in soil and water (Sun et al., 2008). Until now, most studies demonstrated plants response to a single metal. Therefore, studies should focus their combined effects in different plant species. Meharg and Jardine (2003) observed that the interactive toxicity of heavy metals to plants is different from that of an individual heavy metal. They showed that Hg treatment have inhibitory effect on As uptake in rice seedlings. Combined treatment with As and Cd substantially inhibit the uptake and accumulation of Cd by rice seedlings (Sun et al., 2008). Recently, the interactions between Cd and As, Cd and Al, Cd and Cu, Cd and Pb were reported (Shamsi et al., 2008; Sun et al., 2008; Smeets et al., 2009; Xu et al., 2009). However, combined effect of As and Cr have never been reported. Jute is an annual herbaceous plant cultivated for fibers. Cultivated jute varieties belong to the genus of *Corchorus*. The plant grows fast having tall stem and deep penetrating taproot and easily grows in nutrient-poor soil and produces a large amount of valuable biomass. This lingo-cellulose fiber is fully biodegradable (Mir et al., 2008). The plant is also a potential candidate for phytoremediation (Islam et al., 2014). There is scant information available in the literature on the interactions of As and Cr on plants. Hence, the aim of this study is to determine the single and combinatory effects of As and Cr on the growth, oxidative stress and antioxidant enzymes in two jute varieties differing in Cr tolerance.

Results

Plant height and roots length, total biomass of jute grown in Cr and/or As contaminated soil

Plant height, root length and biomass of two jute varieties, O-795 and O-9897 are shown in Fig. 1 A, B and Fig. 2. Exposure of the plants to As inhibited growth in both the varieties, leading to a significant reduction in all examined growth parameters. However, the reduction was more pronounced in O-9897 than O-795. The addition of Cr in soil reduced the growth parameter of the two varieties compared to the control. Cr at low level (100 mg kg^{-1}) did not significantly decrease plant height, root length and total biomass, but at high level (200-400 mg kg^{-1}) these were decreased significantly. Combination of low level (50 mg kg^{-1}) As plus Cr stimulated growth and alleviated Cr stress, but higher level As (100 mg kg^{-1}) plus Cr caused reduction of these growth parameters as compared to the As and Cr stress alone. Apparently, As was more toxic than Cr in jute. Moreover, the variety O-9897 had a greater reduction than variety O-795, in every stress (Fig. 1A, 1B and Fig. 2) suggesting that the variety O-795 was more tolerant under Cr plus As stress than the variety O-9897.

Effect of As and/or Cr on chlorophyll content

Chlorophyll content was measured as SPAD value using a portable SPAD meter. Exposure to As significantly decreased chlorophyll concentration compared with control in both varieties, (Fig. 3). Cr exposed to soil, at low level (100 mg kg^{-1}) had no significant effect on SPAD value but at high level (200 and 400 mg kg^{-1}) a significant reduction of SPAD value was detected for the variety O-795. On the other hand Cr sensitive variety, O-9897, had significantly lower SPAD value than control at all Cr level. Moreover, the effect of Cr treatment on SPAD value of jute varieties under stress varied with variety and Cr level. Combined toxicity of the As plus Cr treatment resulted in a slight increase in SPAD value at low As level (50 mg kg^{-1}) but at high As level (100 mg kg^{-1}) SPAD decreased significantly compared to As and Cr alone which indicate that low level As plus Cr has some beneficial effect on both the tested varieties. Overall, O-795 showed higher SPAD value than O-9897 in all cases.

Effect on chlorophyll florescence and photosynthetic rate

The chlorophyll a fluorescence (Fv/Fm) and photosynthetic rate (Pn) are shown in Fig. 4 A, B. For both the varieties, addition of As significantly decreased Fv/Fm and Pn compared with control (Fig. 4 A, 4 B). Cr exposed to soil, at low level (100 mg kg^{-1}) had no significant effect on Fv/Fm and Pn value but at high level (200, 400 mg kg^{-1}); a significant reduction of all measured parameters could be detected for the variety O-795. On the other hand Cr sensitive variety, O-9897, had significantly lower Fv/Fm and Pn value compared to control at all Cr level. Combined toxicity of the As plus Cr treatment resulted in a slight increase in photosynthetic parameter at low level As (50 mg kg^{-1}) plus Cr, however, decreased at high level As (100 mg kg^{-1}) plus Cr treatment compared to As and Cr alone which indicate that low level As plus Cr has beneficial effect on both varieties. Over all, for all stresses, the highest inhibition of Fv/Fm and Pn was noticed in O-9897, while O-795 was least affected.

Accumulation of As and Cr in roots and leaves

Chromium and As concentration in roots and leaves of two jute varieties are presented in Table 1. The Cr concentration increased dramatically with increasing Cr concentration, being significantly higher in roots than in leaves (Table 1). Addition of As in the soil resulted in a significant increase of As concentration in all plant parts (Table 1). The treatment

Table 1. The effect of arsenic and chromium treatment on the accumulation arsenic and chromium concentration in roots and leaves tissue of two jute varieties.

Variety	As level (mg kg^{-1})	Cr level (mg kg^{-1})	As concentration(mg kg^{-1})		Cr concentration (mg kg^{-1})	
			Root	Leaf	Root	Leaf
O-795	0	0	0.43±0.02i	0.11±0.00i	8.75±0.62m	4.11±0.03n
		100	0.43±0.03i	0.12±0.01i	1006.88±10.49fg	595.27±11.72e
		200	0.48±0.02i	0.15±0.01i	1214.11±7.73e	689.43±16.81cd
		400	0.35±0.04i	0.10±0.01i	1594.33±25.89b	764.17±11.28b
	50	0	44.35±1.46f	12.39±0.19g	11.17±0.72m	5.11±0.05n
		100	55.61±2.54e	22.63±0.09d	1035.22±8.74f	659.66±7.88d
		200	70.52±2.03d	32.93±1.11b	1261.43±16.35d	714.12±13.70b
		400	48.65±2.13f	15.33±0.48g	1653.87±15.55a	767.55±12.35a
	100	0	83.43±1.90c	29.35±0.25d	11.02±0.46m	4.24±0.22n
		100	95.57±1.54b	37.28±0.21c	998.16±26.36g	508.97±10.10f
		200	117.29±1.45a	42.72±0.89a	1189.47±9.98e	605.83±10.49e
		400	69.77±1.97d	23.72±0.29e	1495.89±11.65c	679.56±14.48bc
O-9798	0	0	0.37±0.01i	0.10±0.01i	8.42±0.24m	5.18±0.08n
		100	0.39±0.01i	0.12±0.01i	508.66±15.84k	150.41±9.28lm
		200	0.41±0.01i	0.13±0.01i	739.18±37.87h	263.17±10.94jk
		400	0.33±0.01i	0.09±0.01i	908.72±13.11g	332.17±12.45gh
	50	0	34.51±2.67g	9.87±0.13h	10.39±0.17m	3.79±0.04n
		100	47.52±1.81f	16.48±0.31f	600.78±17.81j	179.54±14.85l
		200	57.43±1.61e	23.64±0.34d	747.37±19.42h	293.49±9.36ij
		400	30.31±1.85g	11.18±0.12h	917.64±13.50g	371.36±12.13g
	100	0	66.43±2.52d	26.26±0.58e	9.11±0.42m	3.50±0.03n
		100	77.49±0.91c	35.76±0.15c	407.66±10.77l	129.19±10.84m
		200	86.94±1.84c	42.00±0.31a	666.58±16.99i	233.39±12.55k
		400	66.31±2.10d	24.49±1.12e	512.37±15.77k	309.38±7.67hi
Interaction			**	**	**	**
Variety and As			**	**	**	**
Variety and Cr			**	NS	**	**
As and Cr			**	**	**	**
Variety + As + Cr			NS	NS	**	NS

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

Table 2. The effect of arsenic and chromium treatment on the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content in leaves and roots of two jute varieties.

Variety	As level (mg kg^{-1})	Cr Level (mg kg^{-1})	MDA (nmol g^{-1} FW)]		H_2O_2 (µmol g^{-1} FW)	
			Root	Leave	Root	Leave
O-795	0	0	29.69l	28.09n	7.50i	12.60ij
		100	33.47j	30.04lmn	8.56i	15.60h
		200	42.36gh	36.22ghij	14.60f	21.72e
		400	47.75de	41.74ef	19.68e	26.62d
	50	0	32.71jk	31.90klm	10.42h	16.21h
		100	30.60kl	25.05o	5.46j	11.61j
		200	37.53i	30.15lmn	8.48i	16.33h
		400	40.13hi	34.71hijk	12.61g	20.17efg
	100	0	38.08i	36.96ghi	15.50f	20.53ef
		100	43.98fg	41.39ef	22.32d	26.48d
		200	49.88cd	47.22c	28.40c	32.50c
		400	55.51b	53.90ab	34.31b	37.54b
O-9897	0	0	32.68jk	28.92mn	10.48h	13.51i
		100	39.50i	34.40ijk	15.70f	19.57fg
		200	45.67ef	39.30fg	22.59d	25.77d
		400	50.53c	43.99de	28.56c	32.49c
		0	37.78i	35.22hijk	15.19f	18.46g
	50	100	34.87j	28.65mn	10.23h	13.53i
		200	40.12hi	32.86jkl	15.73f	19.52fg
		400	45.61ef	37.92gh	22.26d	25.32d
	100	0	43.37fg	41.20ef	22.31d	25.65d
		100	48.33cde	46.26cd	27.51c	32.59c
		200	54.46b	51.10b	32.90b	38.52b
		400	59.67a	56.93a	39.65a	44.36a
Interaction						
Variety and As			NS	NS	*	**
Variety and Cr			NS	NS	**	**
As and Cr			**	**	**	**
Variety+ As+ Cr			NS	NS	**	NS

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

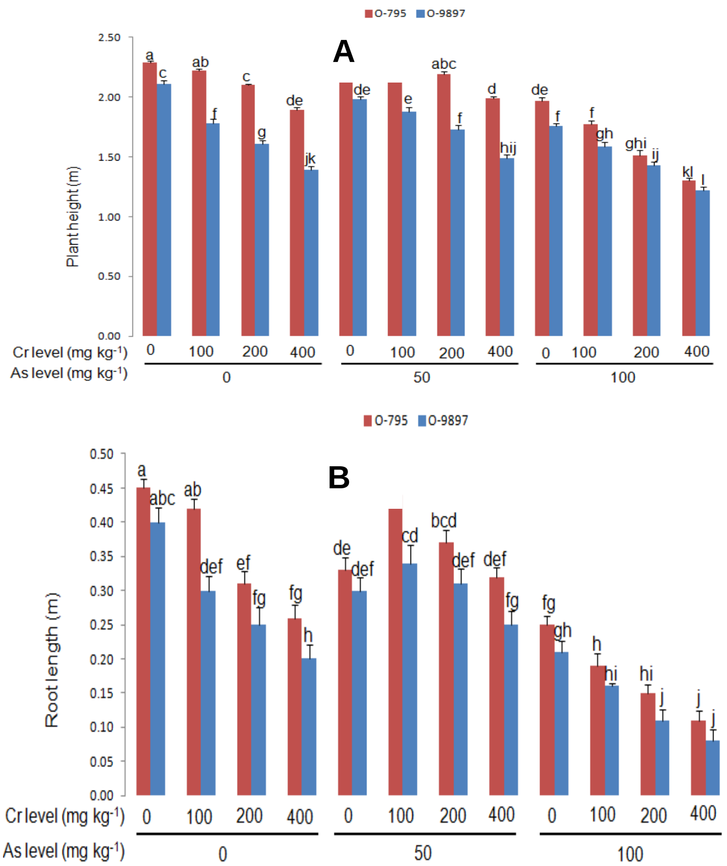

Fig 1. The effect of arsenic and chromium stresses on the plant height (A) and root length (B) of two jute varieties. The same letters after the data indicates that there was no significant difference (p≤ 0.05).

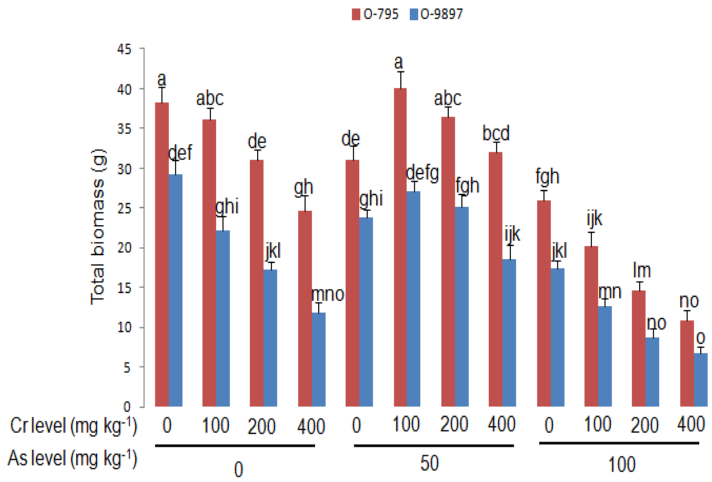

Fig 2. The effect of arsenic and chromium stresses on the total biomass of two jute varieties. The same letters after the data indicates that there was no significant difference (p≤ 0.05).

with low level As (50 mg kg^{-1}) plus Cr significantly increased Cr accumulation in roots and leaves compared with the Cr treatment alone. However, high level (100 mg kg^{-1}) As plus Cr significantly decreased total Cr accumulation compared with the Cr treatment alone and low level As plus Cr in both varieties (Table 1). Moreover, the treatment of As plus low level Cr (100, 200 mg kg^{-1}) significantly increased As level in all plant parts compared with the As treatment alone while high level Cr (400 mg kg^{-1}) plus As significantly decreased As content compared with As alone and As plus low level Cr treatment (Table 1). The effect of As and Cr concentration varied with plant organs, varieties and As, and Cr levels. The increase of As and Cr was more pronounced in ‘O-795’ than in ‘O-9897’ variety.

Lipid peroxidation (MDA) and hydrogen peroxide (H_2O_2) contents

The MDA and H_2O_2 contents in both the roots and leaves are presented in Table 2. For variety O-795, both As and Cr exposure significantly increase the MDA and H_2O_2 content compared with control. The As treatment was more effective in MDA and H_2O_2 content than the Cr treatment. The MDA and H_2O_2 contents in the leaves and roots increased significantly when the plants were exposed to As stress. The addition of Cr to the soil resulted in significant increases in MDA and H_2O_2 contents in both the roots and leaves of the tested varieties. Interestingly MDA and H_2O_2 concentration of the low (50 mg kg^{-1}) As level plus Cr treatment in O-795 was slightly reduced compared to Cr stress alone. On the other hand when the plants were exposed to the combined stress of higher level (100 mg kg^{-1}) As plus Cr, the MDA and H_2O_2 contents were further increased relative to the single stress of either metal. For variety O-9897, Very similar changes were observed in terms of MDA and H_2O_2 concentration for all the treatment (Table 2). However, Cr- or As- induced increment of MDA and H_2O_2 concentration was much higher for variety O-9897 than for variety O-795.

Antioxidant defense response in the two jute varieties

The data for SOD, POD, CAT, GR and APX concentration activity in the leaves and roots of two jute varieties in different treatments are illustrate in Table 3, Table 4 and Fig. 5 A,5 B. For the Cr sensitive variety O-9897, exposure of As or Cr significantly increased SOD, POD, CAT, GR and APX activity compared to control in both leaves and roots. Addition of As significantly increased all measured antioxidant enzymes activities in jute leaves and roots compared with corresponding control. The As treatment was more effective in increasing the activities of tested antioxidant enzymes than Cr treatment. The combined treatment of low As (50 mg kg^{-1}) plus Cr slightly declined the activity of antioxidant enzymes compared with the treatment Cr alone. However, higher level (100 mg kg^{-1}) As plus Cr exposure further increased SOD, POD, CAT, GR and APX activity compared with the treatment of Cr or As alone and low As (50 mg kg^{-1}) plus Cr. By contrast, very similar changes were observed with respect to SOD, POD, CAT, GR and APX concentration in all the treatments in the Cr tolerant variety O-795. Cr tolerant variety, O-795, had consistently lower SOD, POD, MDA and H_2O_2 activities and higher CAT, GR, APX activities than Cr sensitive variety, O-9897.

Table 3. The effect of arsenic and chromium treatment on the superoxide dismutase (SOD) and peroxidase (POD) activities in leaves and roots of two jute varieties.

Variety	As level (mg kg^{-1})	Cr level (mg kg^{-1})	SOD (U g^{-1} FW)		POD [OD 470 g^{-1} FW min^{-1}]	
			Root	Leaf	Root	Leaf
O-795	0	0	111.87o	117.85p	364.20p	99.86p
		100	149.09k	160.83m	408.31n	121.57no
		200	180.34hi	195.63j	542.30h	148.61k
		400	202.86f	238.74f	656.07d	169.67h
	50	0	135.76m	140.64n	392.15o	125.74mn
		100	126.03n	130.47o	386.77o	117.55o
		200	156.18j	154.68m	483.91k	131.70m
		400	184.12h	194.59j	556.11g	150.34jk
	100	0	150.27k	184.68k	445.38l	144.83kl
		100	194.04g	224.51g	520.51i	178.61g
		200	222.71e	260.61d	677.23c	196.04e
		400	259.18b	295.96b	760.08a	244.63b
O-9798	0	0	115.87o	120.85p	356.17p	101.42p
		100	156.78j	167.68l	490.18k	146.55kl
		200	207.12f	215.84h	557.12g	175.46gh
		400	230.13d	249.67e	701.45b	205.42d
	50	0	136.41m	155.86m	394.77o	125.36mno
		100	141.21l	157.67m	421.37m	117.60o
		200	183.67h	168.51l	484.23k	139.58l
		400	204.27f	192.59j	589.31f	156.39ij
	100	0	175.80i	206.46i	507.94j	158.91i
		100	206.04f	234.65f	553.52gh	186.43f
		200	249.00c	278.58c	619.15e	223.60c
		400	274.81a	303.77a	763.28a	253.49a
	Interaction					
	Variety and As		*	NS	**	**
	Variety and Cr		**	**	**	**
	As and Cr		**	**	**	**
	Variety + As + Cr		**	**	**	**

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

Table 4. The effect of arsenic and chromium stresses on the catalase (CAT) and glutathione reductase (GR) activities in leaves and roots of two jute varieties.

Variety	As level (mg kg^{-1})	Cr Level (mg kg^{-1})	CAT (mmol g^{-1} FW)		GR (mmol g^{-1} FW)	
			Root	Leaf	Root	Leaf
O-795	0	0	0.25lmn	0.32kl	0.60lmn	0.45ijk
		100	0.32ijkl	0.35ijk	0.68ijkl	0.51ghij
		200	0.40fgh	0.49de	0.82fgh	0.68ef
		400	0.47de	0.57c	1.07c	0.74de
	50	0	0.34hijk	0.38hij	0.74ghij	0.57gh
		100	0.28km	0.33jkl	0.62klmn	0.47hijk
		200	0.34hijk	0.41gh	0.69ijkl	0.56ghi
		400	0.41efgh	0.49de	0.82fgh	0.65ef
	100	0	0.42efg	0.46ef	0.89def	0.71def
		100	0.51cd	0.53cd	1.05cd	0.81cd
		200	0.60b	0.61b	1.48a	0.94b
		400	0.72a	0.78a	1.65	1.07a
O-9897	0	0	0.21n	0.25m	0.52n	0.39k
		100	0.30jklm	0.33jkl	0.63jklmn	0.48hijk
		200	0.38fghi	0.40hi	0.76ghi	0.62fg
		400	0.45def	0.46ef	0.97cde	0.76de
	50	0	0.31jkl	0.32kl	0.73hijk	0.51ghij
		100	0.24mn	0.29lm	0.56mn	0.43jk
		200	0.29klm	0.35ijk	0.66ijklm	0.53ghij
		400	0.37ghij	0.42fgh	0.81fgh	0.61fg
	100	0	0.41efgh	0.39hi	0.88ef	0.65ef
		100	0.47de	0.45efg	1.00cde	0.74de
		200	0.55bc	0.54c	1.24b	0.87bc
		400	0.61b	0.61b	1.46a	0.96b
	Interaction					
	Variety and As		NS	NS	**	NS
	Variety and Cr		NS	**	**	NS
	As and Cr		**	**	**	**
	Variety + As + Cr		NS	NS	NS	NS

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

Discussion

Both arsenic and chromium are inhibitory to plant growth. However, physiological impacts of their combined effects have never been reported. In the present study, Cr exposure at the low concentration (100 mg kg^{-1}) did not significantly reduce the root length, shoot length and total biomass in Cr tolerant (O-795) variety but all growth parameter was significantly decreased at high concentration (200-400 mg kg^{-1}). However, in case of Cr sensitive variety, (O-9897), all Cr concentration caused significantly reduced all growth parameters. Our result is the good agreement with the previous reports by Yang et al. (2010) in ramie (*Boehmeria nivea)* and by Tiwari et al. (2013) in radish (*Raphanus sativus*), but is different from the finding by Diwan et al. (2010) who reported that Cr treatment ranging from 100 to 400 mg kg^{-1} significantly enhanced the shoot and root biomass of soil-cultured Indian mustard cv. Pusa Jai Kisan. It seems that the effect of Cr on plant growth differ from plant species and culture conditions. However, The Cr tolerant variety, O-795, was less affected than Cr sensitive variety, O-9897. The reduction observed at higher Cr treatments could be due to impaired root growth leading to a reduced uptake of essential nutrient and water and the consequent impact on plant biomass (Barcelo et al., 1985; Chatterjee and Chatterjee, 2000). Although As is a nonessential and highly toxic metal, there is a contradictory role on plant growth depending on As exposure concentrations and plant species. The growth of widely cultivated rice is decreased significantly while As concentrations ranging from 10 to 30 mg kg^{-1} in soil (Azizur et al., 2007). In contrast, As concentrations in soil at $\leq$20 mg kg^{-1} increased the growth of Chinese brake fern (*Pteris vittata* L.). The reasons for this stimulation are not fully known but may be rice is a non-hyperaccumulator of As, on the other hand Chinese brake fern is an As hyperaccumulator. In the present study, the growth parameter of jute variety was significantly reduced when grown in the soil amended with 50-100 mg kg^{-1} As. Jin et al. (2010) observed that growth inhibition was occurred in tall fescue with application of As depending on a degree of concentration. The roots were inhibited to a greater extent than the shoots. This may be due to the higher amount of As in root as most As is concentrated in roots (Shri et al., 2009). The interactive effects of As with Cr on plant growth are contradictory (Oliveira et al., 2014). Interestingly, low level (50 mg kg^{-1}) As plus Cr exposure in the present study significantly alleviated the Cr-induced inhibitory effect on plant growth compared with the Cr treatment alone (Fig. 1A, 1B and Fig. 2). The effect of As on alleviation of Cr toxicity might be attributed partly to the As-induced decreased accumulation of Cr both in roots and in shoots (Oliveira et al., 2014). On the other hand, we observed that the combined stress of high As level (100 mg kg^{-1}) plus Cr caused further reduction of the measured parameters compared to the stress alone. Moreover, more pronounced reduction was observed in Cr sensitive variety. Similarly, it has been reported that As and Cd have a synergistic effect on the inhibition of plant growth in rice (Sun et al., 2008). Moreover, short-term co-exposure of As and Cd in solution culture had synergistic effect on wheat root elongation, in contrast, As and Cd stress in a calcareous soil showed antagonistic effect (Cao et al., 2007). Chlorophyll concentration (SPAD value) is commonly used as an important parameter to evaluate leaf health. In the present study, both As and Cr stress caused a significant reduction in SPAD value except low concentration Cr, and the combined stress of the low As plus Cr increase SPAD value compared to Cr stress alone but high level As plus Cr stress showed

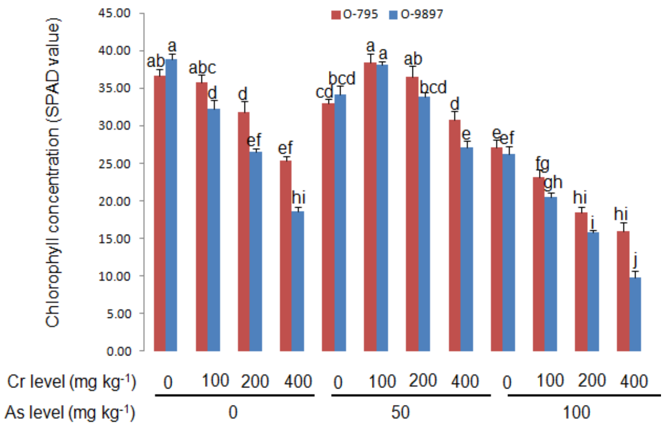

Fig 3. The effect of arsenic and chromium stresses on chlorophyll content of two jute varieties The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

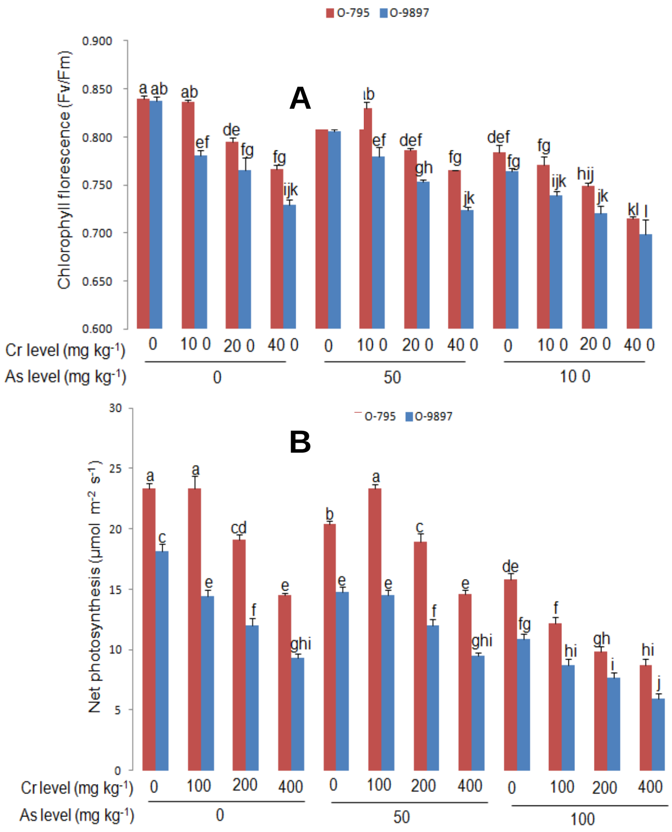

Fig 4. The effect of arsenic and chromium stresses on chlorophyll florescence (A) and photosynthetic rate (B) of two jute varieties. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

further reduction in SPAD value relative to each of these stresses alone. Moreover, O-795 was less affected than O-9897, which indicates a difference between the two jute variety in the effect of As and Cr stresses on SPAD value. Similarly, interaction between Cd and NaCl at higher level decreased plant chlorophyll in maize as compared to Cd and NaCl stress alone (Sepehr and Ghorbanli, 2006). The decrease in growth may be attributed to the reduction in the photosynthetic carbon assimilation. There was a strong linear

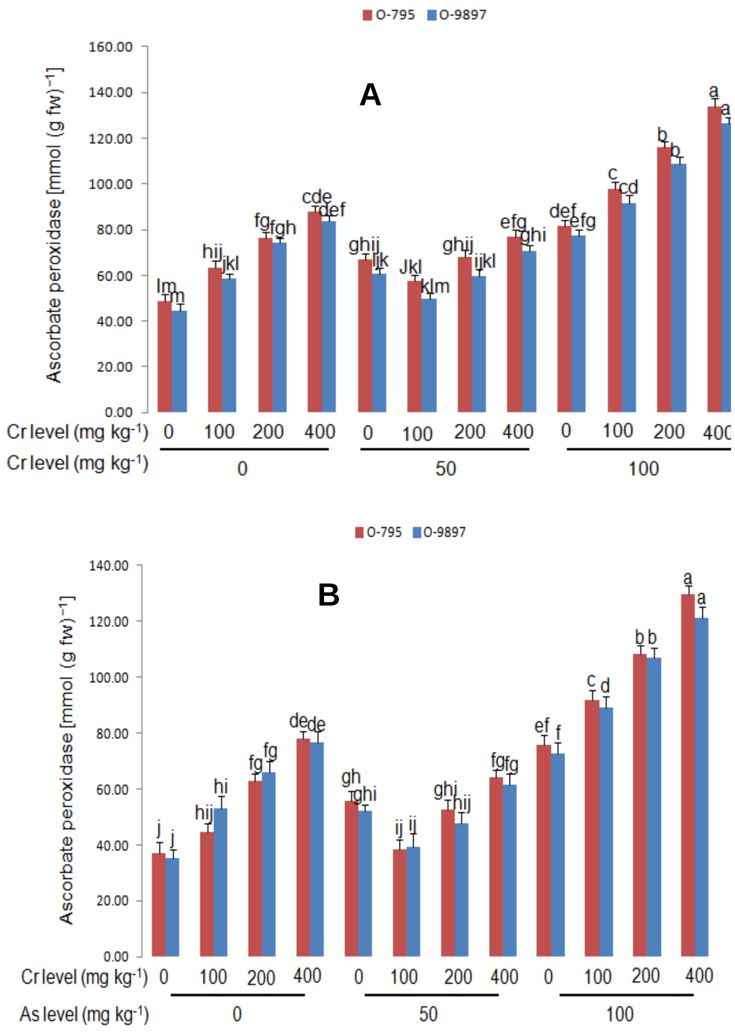

Fig 5. Effect of arsenic and chromium stress on the root ascorbate peroxidase (A) and leaf ascorbate peroxidase (B) activities of two jute varieties. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

relationship between Pn and Fv/Fm. Fv/Fm and Pn decreased significantly with increasing As and Cr concentration except variety O-795 at Cr 100 mg kg^{-1} compared to control. The decline in Pn may be As or Cr stress can affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities (Mateos-Naranjo et al., 2012; Shanker et al., 2005). Paiva et al. (2009) reported the decrease in Pn caused by Cr probably the damage suffered by the photosynthetic system based on the decrease of the maximum quantum efficiency of PSII photochemistry (Fv/Fm). It was also reported that As or Cr had a negative impact on photosynthetic parameter in other plants (Mateos-Naranjo et al., 2012; Subrahmanyam, 2008). We suggest that As and Cr could inhibit the potential activity and the photochemical efficiency of PSII system, which plays an important role in affecting the plant growth traits, such as the plant height, root height, and biomass. The combined stress of the low As plus Cr increase Fv/Fm value compared to Cr stress alone but high level As plus Cr stress showed further reduction in photosynthetic parameter relative to each of these stresses alone. Moreover, O-795 was less affected than O-9897, which indicates a difference between the two jute variety in the effect of As and Cr stresses on Fv/Fm and Pn value. Similarly, the interaction between Al and Cd; Al and Cr can affect photosynthetic parameters more than each metal alone (Shamsi et al., 2008, Ali et al., 2011b). Most probably high level As plus Cr interacts synergistically on Fv/Fm and Pn as observed by Ali et al. (2011b). Arsenic and Cr concentrations in plant tissues increased with increasing As and Cr level (Table 1). The chromium and As concentration in roots was significantly higher than in the leaves, indicating that most metal absorbed in roots and a small proportion translocated into above-ground plant parts. The possible reason Cr in roots could be the immobilization of Cr in the vacuoles of root cells or rapid reduction of Cr^{6+} to Cr^{3+} in cells. Our result showed that jute plant has similar property as of mung bean (Banerjee et al., 2008), barley (Ali et al., 2012) and rice (Zeng et al., 2010). Moreover, it was observed that As was poorly translocated from root to above ground parts (Table 1), (Quanji et al., 2008; Mateos-Naranjo et al., 2012). The interactive effects of metals on their uptake are very complex and vary with metal species (Ali et al., 2012). Interestingly, low level (50 mg kg^{-1}) As plus Cr exposure in the present study significantly increased Cr uptake in all plant parts compared with the Cr treatment alone (Table 1). Our results are in agreements with previous reported results, that revealed Cd and Al had synergistic interaction on uptake of these metals in barley (Guo et al., 2007; Ali et al., 2012) and soybean (Shamsi et al., 2008). On the other hand, we observed that the combined treatment of high As level (100 mg kg^{-1}) plus Cr caused reduction of the measured parameters compared to their single stress alone. Moreover, more pronounced reduction was observed in Cr sensitive variety (Zeng et al., 2010). Therefore, As plus low level Cr (100, 200 mg kg^{-1}) had shown additive or synergistic effects on the uptake of As while As plus high level Cr (400 mg kg^{-1}) showed antagonistic effect. Our results are consistent with the inability of *Pteris vittata* to reduce arsenic in the rhizomes (Mathews et al., 2010). Usually, in plants, toxic metals generate ROS *viz.* hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), hydroxyl radicals (OH^-) and singlet oxygen ($^1O_2$) which are responsible for oxidative stress (Devi and Prasad, 1998). The generated ROS can swiftly attack and damage bio-molecules such as proteins, lipids and nucleic acids. To minimize the deleterious effects of ROS, plants have devolved scavenging system consisting of antioxidant metabolites, such as GSH and enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD). In the present study, increased levels of ROS were found under metal stress in the two jute varieties in terms of MDA and H_2O_2 contents. Moreover, O-9897, a Cr-sensitive variety, had a higher MDA content than O-795, a Cr-tolerant variety, in all stress treatments, indicating less oxidative damage in O-795 compared to O-9897. This result indicates that, the metal-induced ROS had the harmful effect on jute plants. However, the combined stress of low As plus Cr reduced MDA and H_2O_2 content compared to Cr stress alone. Thus it may be assumed that there might be beneficial effect of low As level on the plant growth but it needs to be tested. But at high level As plus Cr caused a further enhancement in MDA and H_2O_2 contents relative to Cr stress alone. Similar results were observed with the interaction of Al and Cr (Ali et al., 2011a), Cd and Pb (Xu et al., 2009) and Cd and Cu (Smeets et al., 2009). Most probably these metals interact synergistically on their uptake and accumulation leading to increased MDA and H_2O_2 contents as was observed by Ali et al. (2011b). Similarly, Oliveira et al. (2014) observed that in *Pteris vittata*, Cr and As had a synergistic interaction upon their uptake. The current results show that both As and Cr stress increase the activities of SOD, POD, APX, CAT and GR in both jute varieties, which is consistent with previous reports (Diwan et al., 2010; Dwivedi et al., 2010). So far this is the first study concerning the effect of the interaction between As and Cr on antioxidant enzymes. Moreover, the extent of antioxidant enzyme activity under stress varies with varieties. The lower antioxidant level in Cr sensitive jute variety, O-9897, agrees with the previous results which showed that Cd sensitive rice and Al sensitive barley suffered severely from damage by Cd plus Al and produced significantly lower biomass than Cd and Al tolerant cultivars (Sun et al., 2008, Ali et al., 2011a). The variety O-9897 had higher SOD and POD activity and lower CAT, APX and GR activity than O-795, implying that the variety O-795 was less susceptible to stress dependent membrane lipid peroxidation. Higher root CAT and GST activities were found in Al-tolerant plants and these enzymes might be vital in alleviating metal stress related oxidative stress (Darko et al., 2004). Even though many enzymes regulate ROS on intracellular levels, but APX, CAT and GR have the most significant role in this regulation (McKersie and Leshem, 1994; Noctor and Foyer, 1998). Therefore, Cr tolerant O-795 had developed a more efficient defense system to mitigate heavy metal induced oxidative stress. The combined stress of low level of As plus Cr treatment caused less inhibition of plant growth and alleviated oxidative stress, in both varieties compared to Cr stress alone. On the other hand, high level As plus Cr caused a further decrease of plant growth and enhancement of MDA and H_2O_2 contents relative to Cr stress alone. These results suggest that both Antagonistic and synergistic effect of As occurs on Cr toxicity based on its concentration.

Materials and Methods

Plant materials

The seeds of the two jute (*Corchorus olitorius* L.) varieties, O-795 and O-9897, were obtained from Bangladesh Jute Research Institute (BJRI), Dhaka, Bangladesh.

Experimental conditions and treatment

The experiment was conducted in a greenhouse at the Gyeongsang National University, Jinju, South Korea; (36° 50' N and 128° 26' E) during the summer season. Seeds of two jute varieties differing in Cr tolerance (tolerant, O-795 and sensitive, O-9897) (Islam et al., 2014) were surface sterilized in 0.5% solution of sodium hypochlorite for 1 min, rinsed with distilled water for 3-4 times following dried on filter paper. After that seeds were directly sown into pots (30 cm×30 cm) filled with a mixture of acid-washed sand and perlite (5:4, v/v), supplemented with different amounts of $K_2Cr_2O_7$ were added to the soil form 4 Cr levels (0, 100, 200 and 400 mg kg^{-1}) and $Na_3AsO_4 \cdot 12H_2O$ to As form 3 levels (0, 50 and 100 mg kg^{-1}). Thus, combination of Cr and As levels resulted in 12 treatments *viz* As 0-Cr 0 mg kg^{-1}, As 0-Cr 100 mg kg^{-1}, As 0-Cr 200 mg kg^{-1}, As 0-Cr 400 mg kg^{-1}, As 50-Cr 0 mg kg^{-1}, As 50-Cr 100 mg kg^{-1}, As 50- Cr 200 mg kg^{-1}, As 50-Cr 400 mg kg^{-1}, As 100-Cr 0 mg kg^{-1}, As 100-Cr 100 mg kg^{-1}, As 100-Cr 200 mg kg^{-1}, As 100-Cr 400 mg kg^{-1}. The pots received natural sunlight and irrigated with drinking water to maintain 60% field capacity. The plants containing the pots were placed in drip trays to prevent any leachate loss and all collected leachate was returned to the respective experimental pots. At every 7th day, the plants were fertilized with 100 mL of Hoagland's nutrient solution (H2395, Sigma, USA). The experiment was arranged as a split-split plot design with jute variety as main plot, As levels as subplot, and Cr levels as sub-subplot; each treatment had three replicates. Two separate experiments *viz*, growth and antioxidant defense activity were analyzed.

Estimation of root length, stem length and total biomass

Sixty days after sowing seed, plants were harvested and the length of the shoots and roots were measured. Roots were washed carefully with 10 mM Na_2EDTA, followed by distilled water to eliminate any residual salt from the surface. Plant parts were separated and dried at 105ºC for 2 h and subsequently at 70ºC for 48 h, until they reached a constant weight. Then total biomass was measured.

Determination of photosynthetic characters

The measurements were carried out on the top most secondary fully expanded leaves. The SPAD Value was measured using a portable chlorophyll meter (Minolta Co. Ltd., Nobi, Japan). The plant leaves were analyzed for photosynthetic activity by monitoring chlorophyll fluorescence (Mini PAM (Walz, Effeltrich, Germany). Fv/Fm (the ratio of variable to maximal fluorescence, which is a measure of the quantum yield of photosystem II photochemistry) values were obtained based on these measurements. Net photosynthetic rate (Pn) was determined using a portable photosynthetic system (LiCor-6400, Nebraska, USA) with an attached LED light source (6400-02B). The measurements were carried out between 10:00 am to 12:00 pm.

Determination of metal concentration in jute plants

The dried root and leaves tissues were ground into powder using a blender (Wonder blender, Osaka chemical Co. Ltd. Japan). The 1 g of each sample was digested with HNO_3-$HClO_4$ (3:1, v/v). The contents of Cr, As and in leaves and roots were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) as described earlier (Islam et al., 2014).

Lipid peroxidation

The lipid peroxidation level in root and leaf tissues was expressed as 2-thiobarbituric acid (TBA) reactive metabolites, mainly malondi-aldehyde (MDA), and was determined according to Ali et al. (2011a).

H_2O_2 accumulation

The hydrogen peroxide (H_2O_2) content was spectrophotometrically determined following the method described by Alam et al. (2010). Briefly, H_2O_2 was extracted by homogenizing 100 mg of leaf or root tissues with 3 mL of phosphate buffer (50 mM, pH 6.8). The homogenate was centrifuged at 6000×g for 25 min. A mixture consisting of 3 mL of extracted solution and 1 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4 was centrifuged at 6000×*g* for 15 min. The supernatant yellow color intensity was measured at 410 nm. The H_2O_2 content was calculated based on the extinction coefficient of 0.28 μmol^{-1} cm^{-1}.

Antioxidant enzymes assay

Sixty days after sowing, the second fully expanded leaves and roots of the plant were collected for enzymatic analysis.

Fresh weighted samples (both leaf and root, 0.5 g) were ground with a mortar and pestle with liquid nitrogen in a homogenization in 0.05 M phosphate buffer (pH 7.8) in an ice bath. The homogenate was centrifuged at 12,000×g for 15 min at 4 °C. The resulting supernatants were stored at 4 °C and used for estimation of various antioxidant enzymes activity. Nitroblue tetrazolium (NBT) method was used for assessing the SOD (EC 1.15.1.1) enzyme activity (Beauchamp and Fridovich, 1971). POD (EC 1.11.1.7) activity was assayed following the method of Putter (1974), with minor modifications. The reaction mixture (3 mL) contains 100 µL enzyme extract, 100 µL guaiacol (1.5%, v/v), 100 µL H_2O_2 (300 mM) and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The absorbance change of the brown guaiacol at 470 nm was recorded for calculating POD activity (ε= 26.6 mM^{-1} cm^{-1}). CAT (EC 1.11.1.6) activity was determined following the method of Aebi (1984). Activity of APX (EC 1.11.1.11) was calculated following the method of Nakano and Asada (1981). The GR (EC 1.6.4.2) activity was determined by the method of Garcia-Limones et al. (2002).

Statistical analysis

Data are presented as the mean of at least three replicates. A two-way aanalysis of variance (ANOVA) was carried out and significant differences of means were compared using the Duncan multiple test (DMRT) using SPSS software (version 12). P value was ≤ 0.05 or 0.01.

Conclusion

In conclusion, Cr and As have distinct interactions that affect growth, oxidative stress and antioxidant enzymes. The combined toxicity of low level arsenic plus chromium is less severe than that of single As or Cr in terms of jute growth. Low level As can mitigate Cr-induced inhibitory effect on plant growth but higher level As plus Cr stress result in a further reduction. Although As or Cr stress induces obvious oxidative damage, the combined stress of low As level plus Cr can alleviated oxidative damage but higher level As plus Cr stress result in a further increase in oxidative stress. The antioxidant defense activity is higher and the lipid peroxidation is lower in the low level As plus Cr treatment than in the Cr treatment alone. The lower combined toxicity of As and Cr in jute might be attributed to the less oxidative stress caused by the interactive effects of As with Cr both in roots and in shoots. The Cr-sensitive variety, O-9897, displays greater oxidative damage than the Cr-tolerant variety, O-795. Therefore, the extent of the oxidative damage induced by toxic metal varies with variety and level of exposure. In case of As and Cr interactive effect on jute, As exposure up to 50 mg/kg is supposed to be useful in mitigating Cr toxicity as As concentration more than 50 mg/kg had the synergistic effect on Cr toxicity. However, why do low levels of As induce antagonistic effect whereas high levels have the synergistic effect on Cr toxicity is need to be explored in future.

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